

10/820,200

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(FILE 'HOME' ENTERED AT 14:39:48 ON 28 JUN 2005)

FILE 'MEDLINE, EMBASE, BIOSIS, BIOTECHDS, SCISEARCH, HCAPLUS, NTIS,
LIFESCI' ENTERED AT 14:40:24 ON 28 JUN 2005

L1 51978 S ALPHA (W) AMYLASE?
L2 15362 S ASPERGILLUS (W) ORYZAE
L3 2086 S L1 AND L2
L4 93 S FUNGAMYL
L5 14 S L3 AND L4
L6 13 DUP REM L5 (1 DUPLICATE REMOVED)
L7 68103 S THERMOSTAB?
L8 92 S L3 AND L7
L9 150853 S DOUGH OR BREW OR BEER OR ALCHOHOL OR MALTOSE
L10 15 S L8 AND L9
L11 11 DUP REM L10 (4 DUPLICATES REMOVED)
L12 811 S L2 AND IMMOBILIZ?
L13 2 S L4 AND L12
E BISGARD-FRANTZEN H/AU
L14 2 S E4
E SVENDSEN A/AU
L15 375 S E3
E PEDERSEN S/AU
L16 1367 S E3
L17 1742 S L14 OR L15 OR L16
L18 5 S L3 AND L17
L19 3 DUP REM L18 (2 DUPLICATES REMOVED)

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| NEWS 4 FEB 28 | BABS - Current-awareness alerts (SDIs) available |
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| NEWS 6 MAR 03 | REGISTRY/ZREGISTRY - Sequence annotations enhanced |
| NEWS 7 MAR 03 | MEDLINE file segment of TOXCENTER reloaded |
| NEWS 8 MAR 22 | KOREPAT now updated monthly; patent information enhanced |
| NEWS 9 MAR 22 | Original IDE display format returns to REGISTRY/ZREGISTRY |
| NEWS 10 MAR 22 | PATDPASPC - New patent database available |
| NEWS 11 MAR 22 | REGISTRY/ZREGISTRY enhanced with experimental property tags |
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| NEWS 16 APR 28 | Improved searching of U.S. Patent Classifications for U.S. patent records in CA/CAplus |
| NEWS 17 MAY 23 | GBFULL enhanced with patent drawing images |
| NEWS 18 MAY 23 | REGISTRY has been enhanced with source information from CHEMCATS |
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| NEWS 20 JUN 06 | The Analysis Edition of STN Express with Discover! (Version 8.0 for Windows) now available |
| NEWS 21 JUN 13 | RUSSIAPAT: New full-text patent database on STN |
| NEWS 22 JUN 13 | FRFULL enhanced with patent drawing images |
| NEWS 23 JUN 20 | MEDICONF to be removed from STN |
| NEWS 24 JUN 27 | MARPAT displays enhanced with expanded G-group definitions and text labels |
| NEWS EXPRESS | JUNE 13 CURRENT WINDOWS VERSION IS V8.0, CURRENT MACINTOSH VERSION IS V6.0c(ENG) AND V6.0Jc(JP), AND CURRENT DISCOVER FILE IS DATED 13 JUNE 2005 |
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FULL ESTIMATED COST 0.21 0.21

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FILE 'LIFESCI' ENTERED AT 14:40:24 ON 28 JUN 2005
COPYRIGHT (C) 2005 Cambridge Scientific Abstracts (CSA)

=> s alpha(w) amylase?
L1 51978 ALPHA(W) AMYLASE?

=> s aspergillus (w)oryzae
L2 15362 ASPERGILLUS (W) ORYZAE

=> s 11 and 12
L3 2086 L1 AND L2

=> s fungamyl
T:4 93 FUNGAMYL

=> s 13 and 14
T.5 14 T.3 AND T.4

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=> dup rem 15  
PROCESSING COMPLETED FOR L5  
L6 13 DUP REM L5 (1 DUPLICATE REMOVED)
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=> d 1-13 ihih ah

L6 ANSWER 1 OF 13 HCPLUS COPYRIGHT 2005 ACS on STN
 ACCESSION NUMBER: 2004:534312 HCPLUS
 DOCUMENT NUMBER: 141:67294
 TITLE: Cloning, purification and characterization of
 thermostable *alpha*-**amylase** from
 Rhizomucor pusillus, and use in liquefying starch,
 production of alcohol, brewing and baking
 INVENTOR(S): Tang, Lan; Wu, Wenping; Duan, Junxin; Johannessen, Pia
 Francke
 PATENT ASSIGNEE(S): Novozymes A/S, Den.
 SOURCE: PCT Int. Appl., 53 pp.
 CODEN: PIXXD2
 DOCUMENT TYPE: Patent
 LANGUAGE: English
 FAMILY ACC. NUM. COUNT: 1
 PATENT INFORMATION:

| PATENT NO. | KIND | DATE | APPLICATION NO. | DATE |
|---------------|---|----------|-----------------|----------|
| WO 2004055178 | A1 | 20040701 | WO 2003-DK882 | 20031216 |
| WO 2004055178 | C2 | 20041007 | | |
| W: | AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW | | | |
| RW: | BW, GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE, SI, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG | | | |

PRIORITY APPLN. INFO.: DK 2002-1928 A 20021217
 AB The present inventors have successfully isolated a gene from *Rhizomucor pusillus* encoding an **alpha**-*amylase* which they have denoted AM782, they have successfully introduced the encoding gene into a recombinant industrial filamentous fungal expression system, and produced the **alpha**-*amylase*. Characterization of the amylase has shown it to be a highly thermoacidophilic **alpha**-*amylase* which has a highly interesting activity as demonstrated by the sugar profile from maltodextrin hydrolysis by amylase AM782. The amylase AM782 can work at a very high temperature, at least up to 70°. The amylase AM782 has a very fast reaction speed; when compared at the same dosage with **Fungamyl** 800 L, the amylase AM782 can achieve in about 3 h, what takes **Fungamyl** 24 to 48 h. Purification and characterization of the **alpha**-*amylase* from *Rhizomucor pusillus* NN046782 is described. Cloning of the gene encoding the AM782 **alpha**-*amylase* of *Rhizomucor pusillus* NN046782 and subcloning and heterologous expression of AM782 amylase is also described. The thermoacidophilic **alpha**-*amylase* of the invention can be used in starch conversion for liquefaction and saccharification, for liquefying starch in a high maltose syrup, for producing alc., for textile desizing, and for brewing and baking.

REFERENCE COUNT: 6 THERE ARE 6 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L6 ANSWER 2 OF 13 HCPLUS COPYRIGHT 2005 ACS on STN
 ACCESSION NUMBER: 2003:997295 HCPLUS
 DOCUMENT NUMBER: 141:102002
 TITLE: Heat inactivation of *Aspergillus oryzae* **alpha**-*amylase* at high and reduced water content
 AUTHOR(S): Samborska, K.; Guiavarc'h, Y.; Van Loey, A. ;

CORPORATE SOURCE: Hendrickx, M.
Laboratory of Food Technology, Department of Food and
Microbial Technology, Katholieke Universiteit Leuven,
Heverlee, B-3001, Belg.

SOURCE: Mededelingen - Faculteit Landbouwkundige en Toegepaste
Biologische Wetenschappen (Universiteit Gent) (2003),
68(3), 247-250

PUBLISHER: CODEN: MFLBER; ISSN: 1373-7503
Universiteit Gent, Faculteit Landbouwkundige en
Toegepaste Biologische Wetenschappen

DOCUMENT TYPE: Journal

LANGUAGE: English

AB The influence of water content on the kinetic parameters of heat inactivation of **Aspergillus oryzae .alpha.-amylase** was studied. Isothermal inactivation kinetics of **Aspergillus oryzae .alpha.-amylase** in both systems followed a first-order model. The influence of water content on the thermal stability of **.alpha.-amylase** was found to be significant. **.alpha.-Amylase** in maltodextrin system at reduced moisture content was much more thermostable than in solution. The temperature range of inactivation in the reduced water content system was 100-115° compared to 62.5-70° for inactivation in aqueous solution. The decrease of water content had also a significant effect on the z-value for thermal inactivation of **Aspergillus oryzae .alpha.-amylase**.

REFERENCE COUNT: 4 THERE ARE 4 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L6 ANSWER 3 OF 13 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN
DUPLICATE 1

ACCESSION NUMBER: 2001-12290 BIOTECHDS

TITLE: New variant of **Fungamyl-like alpha-amylase**, useful for production of maltose syrups, includes mutations that improve stability against heat and acidic pH;
plasmid pTAKA17 expression in bacterium cell for syrup production, dough improvement, brewing and starch liquefaction

AUTHOR: Bisgard-Frantzen H; SvendSen A; Pedersen S

PATENT ASSIGNEE: Novozymes

LOCATION: Bagsvaerd, Denmark.

PATENT INFO: WO 2001034784 17 May 2001

APPLICATION INFO: WO 2000-DK626 10 Nov 2000

PRIORITY INFO: DK 1999-1617 10 Nov 1999

DOCUMENT TYPE: Patent

LANGUAGE: English

OTHER SOURCE: WPI: 2001-367478 [38]

AB A variant (A) of a **Fungamyl-like alpha-amylase** (EC-3.2.1.1) is claimed. (A) has alteration in one of the disclosed amino acid regions. Each alteration is a deletion or substitution of an amino acid an or insertion of an amino acid downstream of a particular position, and (A) retains **alpha-amylase** activity. Also claimed are: DNA construct (II); recombinant expression vector (III); a cell (IV) transformed with the (II) or (III); composition for producing high maltose syrup (HMS) or alcohol; dough improving or brewing composition; producing (M1) of liquefied starch, HMS or alcohol using (A); producing (M2) variants of **Fungamyl-like enzymes** with increased thermostability; production (M3) of (maltose) syrup; and immobilized (A). (A) is used for producing syrups, e.g. of high maltose content, or alcohol from starch, as dough improver for baked goods, in brewing, to increase fermentability of the wort, and for liquefaction of starch. (47pp)

L6 ANSWER 4 OF 13 HCAPLUS COPYRIGHT 2005 ACS on STN
 ACCESSION NUMBER: 1998:475162 HCAPLUS
 DOCUMENT NUMBER: 129:177135
 TITLE: Enzymic degradation of native and acetylated starch-based extruded blends
 AUTHOR(S): Copinet, Alain; Coma, Veronique; Onteniente, Jean Paul; Couturier, Yves
 CORPORATE SOURCE: Groupe Rech. Emballage Produit Alimentaire Compatibilite, Reims, 51686, Fr.
 SOURCE: Packaging Technology & Science (1998), 11(2), 69-81
 CODEN: PTSCEQ; ISSN: 0894-3214
 PUBLISHER: John Wiley & Sons Ltd.
 DOCUMENT TYPE: Journal
 LANGUAGE: English

AB Blends including natural wheat starch and acetylated starch (with substitution degree 1.5) have been extruded so as to obtain a new packaging material. The influence of this extrusion upon the biodegradability of the blends was studied for several acetylated to natural starch ratios both by a colorimetric method (measure of reducing sugars) and by chromatog. anal. (determination of quantities of degradation products).
 The action of a single *alpha*-amylase (*Fungamyl* 800 from *Aspergillus oryzae*) only leads to degradation of the unmodified part of the starch. On the other hand, an acetylesterase (Viscozyme from *Aspergillus niger*) acting in synergy with the same *alpha*-amylase leads to significant degradation of the two major components of the extruded blends. For instance, with 10% acetylated starch 100% of the blend is degraded. The major product of degradation is glucose (97%) because Viscozyme also has α -glucosidase activity. SO, the present study shows the degradable character of this new packaging material even with a high acetylation value.

REFERENCE COUNT: 24 THERE ARE 24 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L6 ANSWER 5 OF 13 HCAPLUS COPYRIGHT 2005 ACS on STN
 ACCESSION NUMBER: 1996:584142 HCAPLUS
 DOCUMENT NUMBER: 125:241792
 TITLE: A method of designing *alpha*-amylase mutants with predetermined properties, *alpha*-amylase variants, and detergents containing the variants
 INVENTOR(S): Svendsen, Allan; Bisgaard-Frantzen, Henrik; Borchert, Torben Vedel
 PATENT ASSIGNEE(S): Novo Nordisk A/s, Den.
 SOURCE: PCT Int. Appl., 171 pp.
 CODEN: PIXXD2
 DOCUMENT TYPE: Patent
 LANGUAGE: English
 FAMILY ACC. NUM. COUNT: 1
 PATENT INFORMATION:

| PATENT NO. | KIND | DATE | APPLICATION NO. | DATE |
|---|------|----------|-----------------|----------|
| WO 9623874 | A1 | 19960808 | WO 1996-DK57 | 19960205 |
| W: AL, AM, AT, AU, AZ, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI | | | | |
| RW: KE, LS, MW, SD, SZ, UG, AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE | | | | |

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|---|----|----------------|-----------------|----------|
| CA 2211316 | AA | 19960808 | CA 1996-2211316 | 19960205 |
| AU 9644834 | A1 | 19960821 | AU 1996-44834 | 19960205 |
| BR 9607013 | A | 19971028 | BR 1996-7013 | 19960205 |
| EP 808363 | A1 | 19971126 | EP 1996-900895 | 19960205 |
| R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, PT, IE | | | | |
| CN 1172501 | A | 19980204 | CN 1996-191745 | 19960205 |
| JP 11500003 | T2 | 19990106 | JP 1996-523187 | 19960205 |
| US 5989169 | A | 19991123 | US 1996-600908 | 19960213 |
| US 6022724 | A | 20000208 | US 1996-683838 | 19960718 |
| US 6440716 | B1 | 20020827 | US 2000-636252 | 20000810 |
| US 2003170769 | A1 | 20030911 | US 2002-184771 | 20020628 |
| US 2005019886 | A1 | 20050127 | US 2004-926720 | 20040826 |
| PRIORITY APPLN. INFO.: | | | | |
| | | DK 1995-128 | A 19950203 | |
| | | DK 1995-1192 | A 19951023 | |
| | | DK 1995-1256 | A 19951110 | |
| | | WO 1996-DK57 | W 19960205 | |
| | | US 1996-600908 | A2 19960213 | |
| | | US 1996-683838 | A1 19960718 | |
| | | US 1999-325603 | B1 19990603 | |
| | | US 1999-327563 | A1 19990608 | |
| | | US 2000-636252 | A1 20000810 | |

AB A method of constructing a variant of a parent Termamyl-like *alpha*-*amylase*, which variant has *alpha*-*amylase* activity and at least one altered property as compared to the parent *alpha*-*amylase*, comprises i) analyzing the structure of the parent Termamyl-like *alpha*-*amylase* to identify at least one amino acid residue or at least one structural part of the Termamyl-like *alpha*-*amylase* (as evaluated on the basis of structural or functional considerations), ii) constructing a Termamyl-like *alpha*-*amylase* variant, which as compared to the parent Termamyl-like *alpha*-*amylase*, has been modified in the amino acid residue or structural part identified in i) so as to alter the property, and iii) testing the resulting Termamyl-like *alpha*-*amylase* variant for the property in question. The resulting Termamyl variants and detergents containing the variants are claimed. [Trp-54]- and [Trp-52, Trp-54]-Termamyl variants were prepared with recombinant *Bacillus subtilis*. Model building had identified these residues as being important for substrate specificity. Alteration of these residues altered the substrate specificity to be more like that of *Fungamyl* (*Aspergillus oryzae* *alpha*-*amylase*).

L6 ANSWER 6 OF 13 HCPLUS COPYRIGHT 2005 ACS on STN
 ACCESSION NUMBER: 1995:998220 HCPLUS
 DOCUMENT NUMBER: 124:120817
 TITLE: Amylase-containing detergent compositions
 INVENTOR(S): Bettoli, Jean-Luc Philippe; Moss, Michael Alan John; Thoen, Christaan Arthur Jacques Kamiel; Boyer, Stanton Lane; Showell, Michael Stanford; Jeffrey, Janice Procter and Gamble Co., USA
 PATENT ASSIGNEE(S): SOURCE: PCT Int. Appl., 40 pp.
 CODEN: PIXXD2
 DOCUMENT TYPE: Patent
 LANGUAGE: English
 FAMILY ACC. NUM. COUNT: 1
 PATENT INFORMATION:

| PATENT NO. | KIND | DATE | APPLICATION NO. | DATE |
|---|------|----------|-----------------|----------|
| WO 9529224 | A1 | 19951102 | WO 1995-US4710 | 19950417 |
| W: AM, AU, BB, BG, BR, BY, CA, CN, CZ, EE, FI, GE, HU, IS, JP, KG, KP, KR, KZ, LK, LR, LT, LV, MD, MG, MN, MX, NO, NZ, PL, RO, RU, SG, SI, SK, TJ, TT, UA, US, UZ, VN | | | | |

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|------------------------|--|----------|-----------------|------------|
| RW: | KE, MW, SD, SZ, UG, AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG | | | |
| CA 2188403 | AA | 19951102 | CA 1995-2188403 | 19950417 |
| AU 9522935 | A1 | 19951116 | AU 1995-22935 | 19950417 |
| EP 756619 | A1 | 19970205 | EP 1995-916433 | 19950417 |
| R: | AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LI, LU, NL, PT, SE | | | |
| CN 1151177 | A | 19970604 | CN 1995-193743 | 19950417 |
| BR 9507397 | A | 19971007 | BR 1995-7397 | 19950417 |
| JP 10501825 | T2 | 19980217 | JP 1995-527702 | 19950417 |
| US 5783546 | A | 19980721 | US 1996-722088 | 19961018 |
| PRIORITY APPLN. INFO.: | | | EP 1994-302878 | A 19940422 |
| | | | WO 1995-US4710 | W 19950417 |

AB A detergent composition comprises an amylase enzyme [50-500 FAU (fungal **alpha-amylase** units)/100 g] which shows CMCase activity (e.g., **Fungamyl**) and/or is an amylase showing a pos. immunol. cross reaction with the antibody of the **Fungamyl** amylase, or an amylase produced by a host organism in which the gene encoding the **Fungamyl** amylase has been cloned. **Fungamyl** is a com. 1,4- α -D-glucan glucano-hydrolase obtained from a strain of **Aspergillus oryzae**, and was previously believed to be inactive in alkaline media.

L6 ANSWER 7 OF 13 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN
ACCESSION NUMBER: 1992-10941 BIOTECHDS

TITLE: Removing cyclodextrin residues from fat and oil;
lipid treatment with **alpha-amylase** or
cyclomaltodextrin-glucanotransferase in an aqueous emulsion

PATENT ASSIGNEE: SKW-Trostberg

PATENT INFO: DE 4041386 25 Jun 1992

APPLICATION INFO: DE 1990-41386 21 Dec 1990

PRIORITY INFO: JP 1990-41386 21 Dec 1990

DOCUMENT TYPE: Patent

LANGUAGE: German

OTHER SOURCE: WPI: 1992-217941 [27]

AB Residues of cyclodextrin are removed from fats and oils (lipids) by emulsifying the lipid in water and enzymatically degrading the cyclodextrin using **alpha-amylase** (EC-3.2.1.1) and/or cyclomaltodextrin-glucanotransferase (EC-2.4.1.19). Cyclodextrin is added to lipid to remove cholesterol, free fatty acids, vitamins and pigments, and its removal is important for use of lipid as food. After enzyme treatment, the residual cyclodextrin content is below 10 ppm. The **alpha-amylase** is derived from *Aspergillus niger*, **Aspergillus oryzae**, *Bacillus polymyxa*, *Bacillus coagulans*, *Flavobacillus* sp. or from pig pancreas, and used at 10-500 U/g cyclodextrin. Cyclomaltodextrin-glucanotransferase is derived from alkalophilic *Klebsiella* or *Micrococcus* spp., and used at 0.5-20 U/g cyclodextrin. Treatment is between the melting point of the lipid and 70 deg (preferably 25-55 deg). In an example, fish oil pretreated with beta-cyclodextrin was emulsified in 1 kg water at 40 deg and pH 5.5, and treated with 50 U **fungamyl** 800 (*A. oryzae alpha-amylase*). After 2 hr, beta-cyclodextrin was undetectable (initially 150 ppm). (3pp)

L6 ANSWER 8 OF 13 HCPLUS COPYRIGHT 2005 ACS on STN

ACCESSION NUMBER: 1991:407379 HCPLUS

DOCUMENT NUMBER: 115:7379

TITLE: Removal of β -cyclodextrin from egg yolk with **alpha-amylase**

INVENTOR(S): Cully, Jan; Vollbrecht, Heinz Ruediger

PATENT ASSIGNEE(S): SKW Trostberg A.-G., Germany

SOURCE: Ger., 3 pp.

DOCUMENT TYPE: Patent
 LANGUAGE: German
 FAMILY ACC. NUM. COUNT: 1
 PATENT INFORMATION:

| PATENT NO. | KIND | DATE | APPLICATION NO. | DATE |
|------------------------|------|----------|-----------------|------------|
| DE 4001611 | C1 | 19910228 | DE 1990-4001611 | 19900120 |
| CA 2029916 | AA | 19910721 | CA 1990-2029916 | 19901114 |
| CA 2029916 | C | 19950912 | | |
| ZA 9009368 | A | 19911030 | ZA 1990-9368 | 19901122 |
| HU 61332 | A2 | 19921228 | HU 1991-37 | 19910108 |
| HU 212921 | B | 19961230 | | |
| JP 04341161 | A2 | 19921127 | JP 1991-3300 | 19910116 |
| FI 9100278 | A | 19910721 | FI 1991-278 | 19910118 |
| PL 166697 | B1 | 19950630 | PL 1991-288756 | 19910118 |
| CZ 279870 | B6 | 19950712 | CZ 1991-127 | 19910121 |
| PRIORITY APPLN. INFO.: | | | DE 1990-4001611 | A 19900120 |

AB β -Cyclodextrin, which is used to remove cholesterol and cholesterol esters from egg yolk by complexation, is subsequently itself removed to a level of <100 ppm by treatment with **alpha-amylase** from Aspergillus niger, A. oryzae, Bacillus polymyxa, B. coagulans, Flavobacterium, or swine pancreas. Thus, 1 kg pretreated egg yolk containing

0.25% β -cyclodextrin was incubated with **Fungamyl** for 2 h at 40° and pH 5.5.

L6 ANSWER 9 OF 13 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN
 ACCESSION NUMBER: 1992-02329 BIOTECHDS
 TITLE: Enzymatic hydrolysis of wheat starch with various amylases;
alpha-amylase
 AUTHOR: Kaprelyants L V; Tarakhtiy L V; Styngach I V
 LOCATION: M. V. Lomonosov Odessky Technological Institute of Food Industry, Odessa, 270039, USSR.
 SOURCE: Biotehnologiya; (1991) 6, 50-52
 CODEN: BTKNEZ

DOCUMENT TYPE: Journal

LANGUAGE: Russian

AB Wheat starch hydrolysis was investigated using the following amylase preparations: (a) Amylosubtilin G10x (3,500 U/g); (b) Amylorizin P10x (1,600 U/g); and (c) **alpha-amylase** (EC-3.2.1.1) **Fungamyl** L from **Aspergillus oryzae** (4,500 U/g). Wheat starch fractions I (20-25 μ m grains) and II (2-5 μ m grains) were produced by sedimentation and centrifugation. Fermentative hydrolysis of wheat starch (15 mg/ml) was performed at pH 6 and varying temperature in a reactor with constant mixing (250 rpm). The carbohydrates composition of the hydrolyzates was determined by liquid chromatography on DEAE-SI 100 and gel filtration on Sephadex G-50. Production of reducing compounds (%) from both wheat fractions I and II increased with time (5-90 min) for Amylosubtilin G10x, Amylorizin P10x and **Fungamyl** 800. A maximum of 32.4% was obtained from fraction I with **Fungamyl** 800 after 90 min. Investigation of the oligosaccharide content of the wheat fraction hydrolyzates revealed the presence of glucose, maltose, maltotriose, maltpentaose, maltohexaose, maltoheptaose and high mol.weight dextrin. (14 ref)

L6 ANSWER 10 OF 13 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN
 ACCESSION NUMBER: 1990-10258 BIOTECHDS

TITLE: Action pattern of **alpha-amylase** from **Aspergillus oryzae** in concentrated media; influence of concentrated maltotetraose solution on activity and specificity

AUTHOR: Graber M; Combes D
LOCATION: Departement de Genie Biochimique et Alimentaire, UA-CNRS 544,
Institut National des Sciences Appliquees, Avenue de
Rangueil, F-31077 Toulouse, France.
SOURCE: Biotechnol.Bioeng.; (1990) 36, 1, 12-18
CODEN: BIBIAU
DOCUMENT TYPE: Journal
LANGUAGE: English

AB **Aspergillus oryzae alpha-amylase**
(EC-3.2.1.1) was purified to homogeneity from **Fungamyl** 800 L (Novo), and its behavior in concentrated solutions of maltotetraose was determined. Substrate inhibition did not occur at 500 g/l (750 mM) maltotetraose concentration. An apparent decrease of hydrolysis rate at this concentration was due to an increase in the number of transglycosylation reactions. These transglycosylation reactions increased with rising substrate concentration from 20 to 200 g/l and from 200 to 500 g/l, although the maximum percentage of oligosaccharides with polymerization degree higher than the starting substrate did not exceed 20% weight/weight. The presence of polyols (water activity depressors), such as

glycerol, xylitol and sorbitol, did not modify the transglycosylation products, but altered the hydrolysis pattern by favoring the formation of low polymerization degree oligosaccharides. This modification pattern might involve, besides direct interactions of polyols with the binding or active site of the enzyme, an indirect effect of the additive on the microenvironment of the protein. (15 ref)

L6 ANSWER 11 OF 13 HCPLUS COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 1983:435068 HCPLUS
DOCUMENT NUMBER: 99:35068
TITLE: Characterization of microbial **alpha-amylases** by analytical determination of the products of starch hydrolysis
AUTHOR(S): Klenz, G.; Krueger, M.; Pantschev, C.; Fabian, G.
CORPORATE SOURCE: Inst. Tech. Mikrobiol., Berlin, Ger. Dem. Rep.
SOURCE: Lebensmittelindustrie (1983), 30(3), 128-30
CODEN: LEINAQ; ISSN: 0024-0028
DOCUMENT TYPE: Journal
LANGUAGE: German
AB The starch degradation products of com. *Bacillus subtilis* **.alpha.-amylases** BAN 240, Amylase 80x, Dexlo 50, and ZF 178; the *B. licheniformis* **.alpha.-amylase**, Termamyl, and the **Aspergillus oryzae** **.alpha.-amylase**, **Fungamyl**, were determined qual. by paper chromatog. and quant. by high-performance liquid chromatog. Both methods allow good separation up to G6 components. Both qual. and quant. similar degradation products were found by examination of the various amylases of *B. subtilis*. However, the quant. pattern of starch degradation products from *B. licheniformis* amylase was different from that of the *B. subtilis* enzymes, and both the qual. and quant. patterns of products from Termamyl were different from those of the bacterial enzymes. The usefulness of these expts. and the methods used in evaluating the optimum application of **.alpha.-amylases** in the brewing industry are discussed.

L6 ANSWER 12 OF 13 HCPLUS COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 1981:402402 HCPLUS
DOCUMENT NUMBER: 95:2402
TITLE: Comparative characterization of **.alpha.-amylase** preparations
AUTHOR(S): Pantschev, C.; Klenz, G.; Haefner, B.
CORPORATE SOURCE: Inst. Enzymol. Tech. Mikrobiol., Berlin, Ger. Dem.

Rep.
SOURCE: Lebensmittelindustrie (1981), 28(2), 71-4
CODEN: LEINAQ; ISSN: 0024-0028
DOCUMENT TYPE: Journal
LANGUAGE: German
AB The simultaneous influence of pH, temperature, substrate, and Ca²⁺ on some .
alpha.-amylase preps. (BAN 240, amylase 80+, Dexlo 50, and .**alpha.-amylase** ZF-178 from *Bacillus subtilis* and **Fungamyl** 800 L from **Aspergillus oryzae**) was analyzed under conditions analogous to those in distilleries. No significant differences were observed between the pH and temperature dependences of preps. from *B. subtilis*. All preps. showed highest

activity at 55-60° and pH 5.5-6.0. In addition to the stabilization provided by Lintner starch in these pH and temperature ranges, an addnl. stabilization by Ca²⁺ was necessary at temps. ≤80° and pH values ≤4.5. **Fungamyl** showed better pH stability but had a low thermal stability. Some differences were observed between hydrolysis products of Lintner starch by bacterial and fungal .**alpha.-amylases** after a reaction period of 3 h. Paper chromatog. anal. showed that the cleavage products due to **Fungamyl** action contained more maltose and fewer long-chain dextrins (>G6) than .**alpha.-amylase** ZF-178 products. After 23 h, bacterial enzyme hydrolysis products still contained a larger portion of long-chain dextrins, less maltose, but more glucose, maltotriose, and maltotetraose than **Fungamyl** products.

L6 ANSWER 13 OF 13 HCPLUS COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 1978:611067 HCPLUS
DOCUMENT NUMBER: 89:211067
TITLE: Degradation of starch granules by .**alpha.-amylases** of fungi
AUTHOR(S): Takaya, T.; Sugimoto, Y.; Imo, E.; Tominaga, Y.; Nakatani, N.; Fuwa, H.
CORPORATE SOURCE: Dep. Food Nutr., Osaka City Univ., Osaka, Japan
SOURCE: Staerke (1978), 30(9), 289-93
CODEN: STRKA6; ISSN: 0038-9056

DOCUMENT TYPE: Journal

LANGUAGE: English

AB The action of 3 preps. of fungal .**alpha.-amylase** (I) on normal corn starch granules and various other types of starch granules was studied. Highly purified I from *Streptomyces hygroscopicus* SF-1084, highly purified **Aspergillus oryzae** I from Biodiastase, and crystalline A. oryzae I from **Fungamyl** 1600 were used. Starch granules attached enzymically were observed by electron scanning microscopy. The attack on corn granules by the 3 enzymes started with small pits on the surface of granules, the pits increased in size and number, and the pores

penetrated into the inner portions toward the center. The optimum pH of degradation was 4.5-5.0 at 37° for 2-h reaction. For corn granules, the main products were maltose and glucose; smaller amts. of higher oligosaccharides were observed throughout the reaction, increasing as the reaction progressed. Maltotriose was not observed at any time. For solid amylase, chromatograms were very similar except for the production of small amts. of maltotriose. For gelatinized amylase, glucose formation was less and increased production of maltotriose and higher oligosaccharides was observed

The relative susceptibility of various types of starch granules to fungal I decreased in the order: waxy corn, normal corn, sweet potato, high-amylose corn, mung bean, and potato.

=> s thermostab?

L7 68103 THERMOSTAB?

=> d his

(FILE 'HOME' ENTERED AT 14:39:48 ON 28 JUN 2005)

FILE 'MEDLINE, EMBASE, BIOSIS, BIOTECHDS, SCISEARCH, HCAPLUS, NTIS, LIFESCI' ENTERED AT 14:40:24 ON 28 JUN 2005

L1 51978 S ALPHA(W)AMYLASE?
L2 15362 S ASPERGILLUS (W)ORYZAE
L3 2086 S L1 AND L2
L4 93 S FUNGAMYL
L5 14 S L3 AND L4
L6 13 DUP REM L5 (1 DUPLICATE REMOVED)
L7 68103 S THERMOSTAB?

=> s 13 and 17

L8 92 L3 AND L7

=> s dough or brew or beer or alchohol or maltose

L9 150853 DOUGH OR BREW OR BEER OR ALCHOHOL OR MALTPOSE

=> s 18 and 19

L10 15 L8 AND L9

=> dup rem 110

PROCESSING COMPLETED FOR L10

L11 11 DUP REM L10 (4 DUPLICATES REMOVED)

=> d 1-11 ibib ab

L11 ANSWER 1 OF 11 HCAPLUS COPYRIGHT 2005 ACS on STN

ACCESSION NUMBER: 2004:534312 HCAPLUS

DOCUMENT NUMBER: 141:67294

TITLE: Cloning, purification and characterization of
thermostable .alpha.-amylase

from Rhizomucor pusillus, and use in liquefying
starch, production of alcohol, brewing and baking

INVENTOR(S): Tang, Lan; Wu, Wenping; Duan, Junxin; Johannessen, Pia
Francke

PATENT ASSIGNEE(S): Novozymes A/S, Den.

SOURCE: PCT Int. Appl., 53 pp.

CODEN: PIXXD2

DOCUMENT TYPE: Patent

LANGUAGE: English

FAMILY ACC. NUM. COUNT: 1

PATENT INFORMATION:

| PATENT NO. | KIND | DATE | APPLICATION NO. | DATE |
|------------------------|---|----------|-----------------|------------|
| WO 2004055178 | A1 | 20040701 | WO 2003-DK882 | 20031216 |
| WO 2004055178 | C2 | 20041007 | | |
| W: | AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW | | | |
| RW: | BW, GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE, SI, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG | | | |
| PRIORITY APPLN. INFO.: | | | DK 2002-1928 | A 20021217 |

AB The present inventors have successfully isolated a gene from Rhizomucor pusillus encoding an **alpha-amylase** which they have denoted AM782, they have successfully introduced the encoding gene into a recombinant industrial filamentous fungal expression system, and produced the **alpha-amylase**. Characterization of the amylase has shown it to be a highly thermoacidophilic **alpha-amylase** which has a highly interesting activity as demonstrated by the sugar profile from maltodextrin hydrolysis by amylase AM782. The amylase AM782 can work at a very high temperature, at least up to 70°. The amylase AM782 has a very fast reaction speed; when compared at the same dosage with Fungamyl 800 L, the amylase AM782 can achieve in about 3 h, what takes Fungamyl 24 to 48 h. Purification and characterization of the **alpha-amylase** from Rhizomucor pusillus NN046782 is described. Cloning of the gene encoding the AM782 **alpha-amylase** of Rhizomucor pusillus NN046782 and subcloning and heterologous expression of AM782 amylase is also described. The thermoacidophilic **alpha-amylase** of the invention can be used in starch conversion for liquefaction and saccharification, for liquefying starch in a high **maltose** syrup, for producing alc., for textile desizing, and for brewing and baking.

REFERENCE COUNT: 6 THERE ARE 6 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L11 ANSWER 2 OF 11 HCPLUS COPYRIGHT 2005 ACS on STN
 ACCESSION NUMBER: 2002:368675 HCPLUS
 DOCUMENT NUMBER: 136:385041
 TITLE: Secondary starch liquefaction in fermentation ethanol production
 INVENTOR(S): Veit, Christopher; Felby, Claus; Fuglsang, Claus Crone
 PATENT ASSIGNEE(S): Novozymes A/S, Den.; Novozymes North America, Inc.
 SOURCE: PCT Int. Appl., 33 pp.
 CODEN: PIXXD2
 DOCUMENT TYPE: Patent
 LANGUAGE: English
 FAMILY ACC. NUM. COUNT: 1
 PATENT INFORMATION:

| PATENT NO. | KIND | DATE | APPLICATION NO. | DATE |
|---|------|----------|-----------------|------------|
| WO 2002038787 | A2 | 20020516 | WO 2001-DK737 | 20011109 |
| WO 2002038787 | A3 | 20020926 | | |
| W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM | | | | |
| RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG | | | | |
| AU 2002013841 | A5 | 20020521 | AU 2002-13841 | 20011109 |
| EP 1335982 | A2 | 20030820 | EP 2001-982195 | 20011109 |
| R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL, TR | | | | |
| US 2004091983 | A1 | 20040513 | US 2003-416393 | 20030509 |
| PRIORITY APPLN. INFO.: | | | DK 2000-1676 | A 20001110 |
| | | | US 2000-252213P | P 20001121 |
| | | | DK 2000-1854 | A 20001211 |
| | | | US 2000-256015P | P 20001215 |
| | | | WO 2001-DK737 | W 20011109 |

AB The invention relates to a method of producing ethanol by fermentation, said method comprising a secondary liquefaction step in the presence of a **thermostable acid alpha-amylase** or, a

thermostable maltogenic acid alpha-amylase.

L11 ANSWER 3 OF 11 EMBASE COPYRIGHT 2005 ELSEVIER INC. ALL RIGHTS RESERVED.
on STN

DUPPLICATE 1

ACCESSION NUMBER: 2002263340 EMBASE
TITLE: Purification and characterisation of amyloytic enzymes
from thermophilic fungus Thermomyces lanuginosus strain
ATCC 34626.
AUTHOR: Nguyen Q.D.; Rezessy-Szabo J.M.; Claeysens M.; Stals I.;
Hoschke A.
CORPORATE SOURCE: A. Hoschke, Department of Brewing, Szent Istvan University,
Menesi ut 45, H-1118 Budapest, Hungary.
hoschke@omega.kee.hu
SOURCE: Enzyme and Microbial Technology, (2 Aug 2002) Vol. 31, No.
3, pp. 345-352.
Refs: 23
ISSN: 0141-0229 CODEN: EMTED2
PUBLISHER IDENT.: S 0141-0229(02)00128-X
COUNTRY: United States
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 004 Microbiology
LANGUAGE: English
SUMMARY LANGUAGE: English
ENTRY DATE: Entered STN: 20020808
Last Updated on STN: 20020808

AB Amyloytic enzymes (**.alpha.-amylase** and glucoamylase)
from Thermomyces lanuginosus ATCC 34626 were purified to electrophoretic
homogeneity. The molecular mass of purified **.alpha.-amylase** and glucoamylase were 61 and 75kDa, respectively. Their
pI values were calculated to be 3.5-3.6 and 4.1-4.3. The amyloytic
enzymes from T. lanuginosus exhibit pH optima in the range 4.6-6.6 in the
case of **.alpha.-amylase** and 4.4-5.6 in the case of
glucoamylase. Both purified enzymes have temperature optima at
70°C. Zn(2+) ions strongly inhibit both enzyme activities. Mn(2+) and
Ba(2+) are activators in the case of glucoamylase; Ca(2+) and
.alpha.-amylase. With half-life times longer than 1 day at 60°C both enzymes prove
to be **thermostable** in the pH range 4.5-8.5. The amyloytic
enzymes from T. lanuginosus loose activities rapidly when incubated at
temperature higher than 80°C or at pH lower than 4.0. Both enzymes
are found to be glycosylated; 8.5% carbohydrate in the case of
.alpha.-amylase and 3.3% in the case of glucoamylase.
The K(m) and V(max) of **.alpha.-amylase** on soluble
starch were 0.68mg/ml and 45.19U/mg, respectively. The K(m) values of
glucoamylase on **maltose**, maltotriose, maltotetraose,
maltpentose and soluble starch were 6.5, 3.5, 2.1, 1.1mM and 0.8mg/ml,
respectively. The first 37 residues of N-terminal of the purified
.alpha.-amylase of T. lanuginosus ATCC 34626 were
sequenced. Almost complete homology with the **.alpha.-amylase**
from **Aspergillus oryzae** and **Emericella nidulans** was observed. ©COPYRGT. 2002 Elsevier Science Inc. All rights
reserved.

L11 ANSWER 4 OF 11 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN
ACCESSION NUMBER: 2001-12290 BIOTECHDS

TITLE: New variant of Fungamyl-like **alpha-amylase**
, useful for production of **maltose** syrups, includes
mutations that improve stability against heat and acidic pH;
plasmid pTAKA17 expression in bacterium cell for syrup
production, dough improvement, brewing and
starch liquefaction
AUTHOR: Bisgard-Frantzen H; SvendSen A; Pedersen S
PATENT ASSIGNEE: Novozymes

LOCATION: Bagsvaerd, Denmark.
PATENT INFO: WO 2001034784 17 May 2001
APPLICATION INFO: WO 2000-DK626 10 Nov 2000
PRIORITY INFO: DK 1999-1617 10 Nov 1999
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2001-367478 [38]

AB A variant (A) of a Fungamyl-like **alpha-amylase** (EC-3.2.1.1) is claimed. (A) has alteration in one of the disclosed amino acid regions. Each alteration is a deletion or substitution of an amino acid an or insertion of an amino acid downstream of a particular position, and (A) retains **alpha-amylase** activity. Also claimed are: DNA construct (II); recombinant expression vector (III); a cell (IV) transformed with the (II) or (III); composition for producing high **malto**se syrup (HMS) or alcohol; **dough** improving or brewing composition; producing (M1) of liquefied starch, HMS or alcohol using (A); producing (M2) variants of Fungamyl-like enzymes with increased **thermostability**; production (M3) of (**malto**) syrup; and immobilized (A). (A) is used for producing syrups, e.g. of high **malto**se content, or alcohol from starch, as **dough** improver for baked goods, in brewing, to increase fermentability of the wort, and for liquefaction of starch. (47pp)

L11 ANSWER 5 OF 11 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation on STN

ACCESSION NUMBER: 1999:317941 SCISEARCH
THE GENUINE ARTICLE: 188EP

TITLE: Thermodynamic stability of a cold-active **alpha-**
amylase from the Antarctic bacterium Alteromonas
haloplanctis

AUTHOR: Feller G (Reprint); dAmico D; Gerday C

CORPORATE SOURCE: UNIV LIEGE, INST CHEM B6, BIOCHEM LAB, B-4000 LIEGE,
BELGIUM (Reprint)

COUNTRY OF AUTHOR: BELGIUM

SOURCE: BIOCHEMISTRY, (6 APR 1999) Vol. 38, No. 14, pp. 4613-4619.
Publisher: AMER CHEMICAL SOC, 1155 16TH ST, NW,
WASHINGTON, DC 20036.

ISSN: 0006-2960.

DOCUMENT TYPE: Article; Journal

FILE SEGMENT: LIFE

LANGUAGE: English

REFERENCE COUNT: 48

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB The thermal stability of the cold-active **alpha-**
amylase (AHA) secreted by the Antarctic bacterium Alteromonas
haloplanctis has been investigated by intrinsic fluorescence, circular
dichroism, and differential scanning calorimetry. It was found that this
heat-labile enzyme is the largest known multidomain protein exhibiting a
reversible two-state unfolding, as demonstrated by the recovery of Delta
H-cal values after consecutive calorimetric transitions, a Delta
H-cal/Delta H-eff ratio close to unity, and the independence of unfolding
thermodynamic parameters of scan rates. By contrast, the mesophilic
alpha-amylases investigated here (from porcine pancreas,
human salivary glands, yellow meal beetle, *Bacillus amyloliquefaciens*, and
Bacillus licheniformis) unfold irreversibly according to a non-two-state
mechanism. Unlike mesophilic **alpha-amylases**, the
melting point of AHA is independent of calcium and chloride binding while
the allosteric and structural functions of these ions are conserved. The
thermostability of AHA at optimal conditions is characterized by a
T-m of 43.7 degrees C, a Delta H-cal of 238 kcal mol(-1), and a Delta C-p
of 8.47 kcal mol(-1) K-1. These values were used to calculate the Gibbs
free energy of unfolding over a wide range of temperatures. This stability
curve shows that (a) the specific Delta G(max) of AHA [22 cal (mol of

residue) (-1)] is 4 times lower than that of mesophilic **alpha-amylases**, (b) group hydration plays a crucial role in the enzyme flexibility at low temperatures, (c) the temperature of cold unfolding closely corresponds to the lower limit of bacterial growth, and (d) the recombinant heat-labile enzyme can be expressed in mesophilic hosts at moderate temperatures. It is also argued that the cold-active **alpha-amylase** has evolved toward the lowest possible conformational stability of its native state.

L11 ANSWER 6 OF 11 HCPLUS COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 1990:587055 HCPLUS
DOCUMENT NUMBER: 113:187055
TITLE: Action pattern and substrate specificity of a **thermostable .alpha.-amylase**
from *Bacillus apiarius* CBML 152
AUTHOR(S): Ghosh, S. B.; Chandra, A. K.
CORPORATE SOURCE: Dep. Bot., Univ. Calcutta, Calcutta, 700 019, India
SOURCE: Annali di Microbiologia ed Enzimologia (1989), 39(Pt. 2), 195-202
CODEN: AMEZAB; ISSN: 0003-4649
DOCUMENT TYPE: Journal
LANGUAGE: English
AB A **thermostable amylase** was purified from a strain of *B. apiarius* CBML 152 and the enzyme was determined to be as an **.alpha.-amylase** (EC 3.2.1.1; α -1,4-glucan-4-glucanohydrolase). The enzyme could bypass the α -1,6-linkages at branch points and could hydrolyze the starchy substrates completely. The enzyme was a saccharifying type of **.alpha.-amylase** and produced more than 95% reducing sugars as glucose (G1) and **maltose** (G2), along with maltotriose (G3). No maltotetraose was produced. To an extent, the enzyme could hydrolyze the α -1,6-branch points and showed very broad substrate specificity. Kinetic studies revealed that the enzyme had affinity towards both straight chain (amylose- V_m = 66.6 U/mL and K_m = 5.8 mg/mL) and branched chain (amylopectin V_m = 71.4 U/mL and K_m = 5.0 mg/mL) substrates. The velocity of the enzyme activity, for hydrolysis and sugar production, was very high.

L11 ANSWER 7 OF 11 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN
ACCESSION NUMBER: 1987-12537 BIOTECHDS
TITLE: The biotechnological relevance of starch-degrading enzymes; analysis of e.g. **thermostable alpha-amylase**; ethanol production etc.
AUTHOR: Stewart G G
CORPORATE SOURCE: Labatt-Brewing
LOCATION: Production Research Department, Labatt Brewing Company Ltd., London, Ontario, Canada.
SOURCE: Critical Rev.Biotechnol.; (1987) 5, 2, 89-93
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Starch-degrading enzymes of actual or potential industrial importance may be classified into 6 classes with respect to bond hydrolysis. The enzymes described include **alpha-amylases** (EC-3.2.1.1), beta-amylases (EC-3.2.1.2), glucoamylases (EC-3.2.1.3), pullulanase (EC-3.2.1.41) and alpha-glucosidase (EC-3.2.1.20). The commercial use of **thermostable alpha-amylases** is considered, with reference to the enzyme produced by *Bacillus amyloliquefaciens* and *Bacillus licheniformis*, and to the production of high **maltose** syrups by *Aspergillus oryzae alpha-amylase*. The enzymatic hydrolysis of starch to fermentable sequences is a coordinated system involving a number of amyloytic enzymes. Future developments with thermophilic amyloytic microorganisms will lead to improvements in enzyme and ethanol production. Ethanol-tolerant mutants of *Clostridium thermohydrosulfuricum* and

Clostridium thermocellum have been isolated. Future targets will be the selection of ethanol-tolerant, high-yield mutants of amylolytic strains. (8 ref)

L11 ANSWER 8 OF 11 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN
ACCESSION NUMBER: 1986-10226 BIOTECHDS
TITLE: Studies on the application of maltogenic amylase in the production of **maltose** containing syrup; use in combination with pullulanase and fungal **alpha-amylase**
AUTHOR: Slominska L; Starogardzka G
LOCATION: Central Laboratorium Przemyslu Ziemniaczanego, Zwierzniecka 18, 60-814 Poznan, Poland.
SOURCE: Starch; (1986) 38, 6, 205-10
CODEN: STARDD
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The **thermostable** and relatively acid stable maltogenic amylase produced by *Bacillus stearothermophilus* was studied, during an analysis of the advantages of using a maltogenic amylase for **maltose** production during saccharification. Experiments were performed using *B. stearothermophilus* maltogenic amylase SP 295, with Polish potato starch as the substrate. A slurry of the starch was subjected to liquefaction at 85 deg for 1 hr with *Bacillus subtilis* **alpha-amylase** (EC-3.2.1.1) (Amylogal CS). The pH was adjusted to 5.0-5.3 and the temperature raised to 105 deg for 15-30 min. Spray-dried maltodextrin was redissolved and saccharified using maltogenic amylase, *Bacillus* sp. pullulanase (EC-3.2.1.41) and fungal (*Aspergillus oryzae*) **alpha-amylase** at 60 deg for 72 hr. With the maltogenic amylase, potato syrup containing 70-80% **maltose** was obtained from DE 12 enzyme liquefied starch at a concentration of 30-35%. A combination of the 3 saccharifying enzymes gave 85% **maltose**. Maltogenic amylase used with pullulanase increased the **maltose** yield and decreased saccharification time. (10 ref)

L11 ANSWER 9 OF 11 HCPLUS COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 1980:72338 HCPLUS
DOCUMENT NUMBER: 92:72338
TITLE: Degradation of elsinan by **.alpha.-amylases**
AUTHOR(S): Tsumuraya, Yoichi; Misaki, Akira
CORPORATE SOURCE: Fac. Sci. Living, Osaka City Univ., Osaka, 558, Japan
SOURCE: Journal of Applied Biochemistry (1979), 1(3), 235-46
CODEN: JABIDV; ISSN: 0161-7354
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Elsinan, a new α -D-glucan consisting of maltotriose and maltotetraose units joined by α -(1 \rightarrow 3)-D-glucosidic linkages was degraded by several **.alpha.-amylases**, e.g., salivary, hog pancreatic, *Aspergillus oryzae*, and *Bacillus subtilis* saccharifying **.alpha.-amylase**. The action of human salivary **.alpha.-amylase** on elsinan resulted in release of O- α -D-glucopyranosyl-(1 \rightarrow 3)-O- α -D-glucopyranosyl-(1 \rightarrow 4)-D-glucopyranose as a major product together with **maltose** and O- α -D-glucopyranosyl-(1 \rightarrow 4)-O- α -D-glucopyranosyl-(1 \rightarrow 3)-O- α -D-glucopyranosyl-(1 \rightarrow 4)-D-glucopyranose. It is proposed that α -D-glucosidic linkages involving the hydroxyl group at the C-4 position of glucose units whose 1-positions are involved in α -(1 \rightarrow 4)-D-glucosidic linkages are preferentially attacked by human salivary **.alpha.-amylase**. *B. subtilis* Liquefying **.alpha.-amylase**, a **thermostable** bacterial **.alpha.-amylase**,

β -amylase, and glucoamylase did not hydrolyze elsinan. The substrate specificities of α -amylases are discussed in relation to their ability to hydrolyze elsinan and the significance of the findings in relation to the application of elsinan as a food additive and pharmaceutical ingredient is considered.

L11 ANSWER 10 OF 11 HCPLUS COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 1954:19673 HCPLUS
DOCUMENT NUMBER: 48:19673
ORIGINAL REFERENCE NO.: 48:3580g-i,3581a-f
TITLE: The use of fungal enzymes for breadmaking purposes
AUTHOR(S): Greup, D. H.; Hintzer, H. M. R.
CORPORATE SOURCE: Central Instituut Voor Voedingsonderzoek T.N.O., Wageningen, The Netherlands
SOURCE: 2nd Intern. Congr. Fermentation Inds. Knocke, Lectures and Communs. (1952) 232-338
DOCUMENT TYPE: Journal
LANGUAGE: Unavailable

AB The need for maintaining an optimum concentration of α - and β -amylase in the process of breadmaking and the suitability of fungal enzyme preps. for this purpose are discussed. α - and β -Amylases together act on starch and bring about a rapid saccharification which provides the fermentable sugar for the yeast. A deficiency of α -amylase limits saccharification and makes the gas production insufficient in the final stages. This deficiency of normal sound flour can be avoided by using flour from sprouted wheat, but owing to its excessive content of dextrins, this has the advantage of making the dough and bread crumbs sticky. The long-employed alternative is to supplement the flour with malt-enzyme preps., but the use of enzyme preps. from several molds, such as certain strains of *Aspergillus oryzae*, is recently receiving considerable interest. Some characteristic properties of the crystalline fungal α -amylase, prepared by fractionation with $(NH_4)_2SO_4$, are lack of thermostability, stability in the cold between pH 4.7 and 7.8, isoelec. point at about 4.0, and nondependence on any ions such as Ca^{++} for its activity. The effect, on the quality of Dutch white bread, of the use of 2 fungal enzyme preps., Diastase 33 (I) and Rhozyme-S (II), is studied, the former being highly amylolytic and poorly proteolytic while the latter is a highly amylolytic and a highly proteolytic preparation. The results showed that these preps. when used at suitable levels improved the quality of the bread, while excessive use was detrimental. The results from baking tests were: (1) Dough consistency appeared to decrease and dough-handling properties improved. This effect was greater in the case of II, since for I the amount of susceptible starch was a limiting factor, while II was not limited by the nature of the gluten substrate. (2) Bread properties such as the color of the crust, loaf volume, and crumb characteristics improved. Crumb compressibility at different storage times was determined by using a panimeter and this showed that softness of the bread had increased. Similarly carried out studies showed that, owing to the effect of I and II, the maltose value was raised only slightly while gas production, measured over a period of several hrs., was increased considerably, I being less effective than II. It is suggested that increased gas production, which becomes more pronounced under the action of heat during the first half of the baking process, contributes to better oven spring and improved loaf volume. The maximum paste viscosity (measured with a Brabender Amylograph) was hardly affected by the fungal enzymes because of their low inactivation temps. Thus, it is claimed that treatment with fungal enzymes permits the formation of sugars without any appreciable decrease in the viscosity of gelatinized starch. Also, the formation of dextrins at elevated temps. will be held at a min. and the choice of the enzyme level may be less critical than for malt α -amylase, which has a relatively high inactivation temperature. Other suggested advantages are

increased availability and mild degradation conditions of starch and liberation of bound β -amylase, which increase the rate of starch hydrolysis and gas production. The presence of other factors not included in this study, e.g. the quality of susceptible starch, nature of starch granules and gluten proteins, α -amylase content of flour, influence of proteolytic enzymes on bound enzymes, etc. may, of course, influence the response of the flour to fungal enzymes.

L11 ANSWER 11 OF 11 HCPLUS COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 1954:19674 HCPLUS
DOCUMENT NUMBER: 48:19674
ORIGINAL REFERENCE NO.: 48:3580g-i,3581a-f
TITLE: The use of fungal enzymes for breadmaking purposes
AUTHOR(S): Greup, D. H.; Hintzer, H. M. R.
CORPORATE SOURCE: Central Instituut Voor Voedingsonderzoek T.N.O., Wageningen, Neth.
SOURCE: Central Inst. Voedingsonderzoek T.N.O. Afdel. Graan-, Meel-en Broodonderzoek Wageningen, Mededel (1952), No. 44E,
DOCUMENT TYPE: Journal
LANGUAGE: Unavailable

AB The need for maintaining an optimum concentration of α - and β -amylase in the process of breadmaking and the suitability of fungal enzyme preps. for this purpose are discussed. α - and β -Amylases together act on starch and bring about a rapid saccharification which provides the fermentable sugar for the yeast. A deficiency of α -amylase limits saccharification and makes the gas production insufficient in the final stages. This deficiency of normal sound flour can be avoided by using flour from sprouted wheat, but owing to its excessive content of dextrins, this has the advantage of making the dough and bread crumbs sticky. The long-employed alternative is to supplement the flour with malt-enzyme preps., but the use of enzyme preps. from several molds, such as certain strains of *Aspergillus oryzae*, is recently receiving considerable interest. Some characteristic properties of the crystalline fungal α -amylase, prepared by fractionation with $(NH_4)_2SO_4$, are lack of thermostability, stability in the cold between pH 4.7 and 7.8, isoelec. point at about 4.0, and nondependence on any ions such as Ca^{++} for its activity. The effect, on the quality of Dutch white bread, of the use of 2 fungal enzyme preps., Diastase 33 (I) and Rhozyme-S (II), is studied, the former being highly amylolytic and poorly proteolytic while the latter is a highly amylolytic and a highly proteolytic preparation. The results showed that these preps. when used at suitable levels improved the quality of the bread, while excessive use was detrimental. The results from baking tests were: (1) Dough consistency appeared to decrease and dough-handling properties improved. This effect was greater in the case of II, since for I the amount of susceptible starch was a limiting factor, while II was not limited by the nature of the gluten substrate. (2) Bread properties such as the color of the crust, loaf volume, and crumb characteristics improved. Crumb compressibility at different storage times was determined by using a panimeter and this showed that softness of the bread had increased. Similarly carried out studies showed that, owing to the effect of I and II, the maltose value was raised only slightly while gas production, measured over a period of several hrs., was increased considerably, I being less effective than II. It is suggested that increased gas production, which becomes more pronounced under the action of heat during the first half of the baking process, contributes to better oven spring and improved loaf volume. The maximum paste viscosity (measured with a Brabender Amylograph) was hardly affected by the fungal enzymes because of their low inactivation temps. Thus, it is claimed that treatment with fungal enzymes permits the formation of sugars without any appreciable decrease in the viscosity of gelatinized starch. Also, the formation of dextrins at elevated temps.

will be held at a min. and the choice of the enzyme level may be less critical than for malt **.alpha.-amylase**, which has a relatively high inactivation temperature. Other suggested advantages are increased availability and mild degradation conditions of starch and liberation of bound β -amylase, which increase the rate of starch hydrolysis and gas production. The presence of other factors not included in this study, e.g. the quality of susceptible starch, nature of starch granules and gluten proteins, **.alpha.-amylase** content of flour, influence of proteolytic enzymes on bound enzymes, etc. may, of course, influence the response of the flour to fungal enzymes.

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(FILE 'HOME' ENTERED AT 14:39:48 ON 28 JUN 2005)

FILE 'MEDLINE, EMBASE, BIOSIS, BIOTECHDS, SCISEARCH, HCAPLUS, NTIS, LIFESCI' ENTERED AT 14:40:24 ON 28 JUN 2005

L1 51978 S ALPHA (W) AMYLASE?
L2 15362 S ASPERGILLUS (W) ORYZAE
L3 2086 S L1 AND L2
L4 93 S FUNGAMYL
L5 14 S L3 AND L4
L6 13 DUP REM L5 (1 DUPLICATE REMOVED)
L7 68103 S THERMOSTAB?
L8 92 S L3 AND L7
L9 150853 S DOUGH OR BREW OR BEER OR ALCOHOL OR MALTOSE
L10 15 S L8 AND L9
L11 11 DUP REM L10 (4 DUPLICATES REMOVED)

=> s l2 and immobiliz?

L12 811 L2 AND IMMOBILIZ?

=> s l4 and l12

L13 2 L4 AND L12

=> d 1-2 ibib ab

L13 ANSWER 1 OF 2 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN
ACCESSION NUMBER: 2001-12290 BIOTECHDS
TITLE: New variant of **Fungamyl**-like alpha-amylase, useful
for production of maltose syrups, includes mutations that
improve stability against heat and acidic pH;
plasmid pTAKA17 expression in bacterium cell for syrup
production, dough improvement, brewing and starch
liquefaction

AUTHOR: Bisgard-Frantzen H; SvendSen A; Pedersen S

PATENT ASSIGNEE: Novozymes

LOCATION: Bagsvaerd, Denmark.

PATENT INFO: WO 2001034784 17 May 2001

APPLICATION INFO: WO 2000-DK626 10 Nov 2000

PRIORITY INFO: DK 1999-1617 10 Nov 1999

DOCUMENT TYPE: Patent

LANGUAGE: English

OTHER SOURCE: WPI: 2001-367478 [38]

AB A variant (A) of a **Fungamyl**-like alpha-amylase (EC-3.2.1.1) is claimed. (A) has alteration in one of the disclosed amino acid regions. Each alteration is a deletion or substitution of an amino acid and or insertion of an amino acid downstream of a particular position, and (A) retains alpha-amylase activity. Also claimed are: DNA construct (II); recombinant expression vector (III); a cell (IV) transformed with the (II) or (III); composition for producing high maltose syrup (HMS) or alcohol; dough improving or brewing composition; producing (M1) of

liquefied starch, HMS or alcohol using (A); producing (M2) variants of **Fungamyl**-like enzymes with increased thermostability; production (M3) of (maltose) syrup; and immobilized (A). (A) is used for producing syrups, e.g. of high maltose content, or alcohol from starch, as dough improver for baked goods, in brewing, to increase fermentability of the wort, and for liquefaction of starch. (47pp)

L13 ANSWER 2 OF 2 HCPLUS COPYRIGHT 2005 ACS on STN

ACCESSION NUMBER: 2001:360158 HCPLUS

DOCUMENT NUMBER: 134:363353

TITLE:

Fungamyl-like Aspergillus

oryzae α -amylase variants with improved

thermal stability and applications to starch processes

INVENTOR(S): Bisgard-Frantzen, Henrik; Svendsen, Allan; Pedersen, Sven

PATENT ASSIGNEE(S): Novozymes A/S, Den.

SOURCE: PCT Int. Appl., 48 pp.

CODEN: PIXXD2

DOCUMENT TYPE: Patent

LANGUAGE: English

FAMILY ACC. NUM. COUNT: 1

PATENT INFORMATION:

| PATENT NO. | KIND | DATE | APPLICATION NO. | DATE |
|---|------|----------|-----------------|-------------|
| WO 2001034784 | A1 | 20010517 | WO 2000-DK626 | 20001110 |
| W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM | | | | |
| RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG | | | | |
| AU 2001012696 | A5 | 20010606 | AU 2001-12696 | 20001110 |
| EP 1230351 | A1 | 20020814 | EP 2000-974351 | 20001110 |
| R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL, TR | | | | |
| JP 2003513666 | T2 | 20030415 | JP 2001-537481 | 20001110 |
| US 2004229764 | A1 | 20041118 | US 2004-820200 | 20040407 |
| PRIORITY APPLN. INFO.: | | | DK 1999-1617 | A 19991110 |
| | | | US 1999-165786P | P 19991116 |
| | | | US 2000-710339 | A1 20001109 |
| | | | WO 2000-DK626 | W 20001110 |

AB The invention relates to a variant of a parent **Fungamyl**-like fungal α -amylase, which exhibits improved thermal stability at acidic pH suitable for, e.g., starch processes. Cloning, amino acid and encoding nucleotide sequences, and mutagenesis of α -amylase from **Aspergillus oryzae** are provided. Construction of variant Q153S α -amylase (Q173S pre- α -amylase) from *A. oryzae* is disclosed.

REFERENCE COUNT: 8 THERE ARE 8 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

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FILE 'MEDLINE, EMBASE, BIOSIS, BIOTECHDS, SCISEARCH, HCPLUS, NTIS, LIFESCI' ENTERED AT 14:40:24 ON 28 JUN 2005

L1 51978 S ALPHA(W)AMYLASE?

L2 15362 S ASPERGILLUS (W) ORYZAE
L3 2086 S L1 AND L2
L4 93 S FUNGAMYL
L5 14 S L3 AND L4
L6 13 DUP REM L5 (1 DUPLICATE REMOVED)
L7 68103 S THERMOSTAB?
L8 92 S L3 AND L7
L9 150853 S DOUGH OR BREW OR BEER OR ALCHOHOL OR MALTOSE
L10 15 S L8 AND L9
L11 11 DUP REM L10 (4 DUPLICATES REMOVED)
L12 811 S L2 AND IMMOBILIZ?
L13 2 S L4 AND L12

=> e bisgard-frantzen h/au
E1 1 BISGARD P/AU
E2 1 BISGARD POUL/AU
E3 0 --> BISGARD-FRANTZEN H/AU
E4 2 BISGARDFRANTZEN H/AU
E5 1 BISGAWA F/AU
E6 2 BISGAY K/AU
E7 1 BISGAY L/AU
E8 6 BISGEIER G/AU
E9 10 BISGEIER G P/AU
E10 1 BISGEIER GEORGE/AU
E11 2 BISGES A/AU
E12 16 BISGES A D/AU

=> s e4
L14 2 "BISGARDFRANTZEN H"/AU

=> e svendsen a/au
E1 1 SVENDSE F/AU
E2 6 SVENDSEN/AU
E3 375 --> SVENDSEN A/AU
E4 1 SVENDSEN A A/AU
E5 363 SVENDSEN A B/AU
E6 109 SVENDSEN A BAERHEIM/AU
E7 1 SVENDSEN A BARHEIM/AU
E8 17 SVENDSEN A J/AU
E9 12 SVENDSEN A K/AU
E10 1 SVENDSEN A L/AU
E11 4 SVENDSEN A M/AU
E12 3 SVENDSEN A M B/AU

=> s e3
L15 375 "SVENDSEN A"/AU

=> e pedersen s/au
E1 1 PEDERSEN RUNE/AU
E2 1 PEDERSEN RUTH L/AU
E3 1367 --> PEDERSEN S/AU
E4 4 PEDERSEN S */AU
E5 553 PEDERSEN S A/AU
E6 7 PEDERSEN S A S/AU
E7 1 PEDERSEN S ANKER/AU
E8 402 PEDERSEN S B/AU
E9 1 PEDERSEN S BOEL/AU
E10 64 PEDERSEN S C/AU
E11 15 PEDERSEN S D/AU
E12 185 PEDERSEN S E/AU

=> s e3
L16 1367 "PEDERSEN S"/AU

=> s l14 or l15 or l16
L17 1742 L14 OR L15 OR L16

=> d his

(FILE 'HOME' ENTERED AT 14:39:48 ON 28 JUN 2005)

FILE 'MEDLINE, EMBASE, BIOSIS, BIOTECHDS, SCISEARCH, HCAPLUS, NTIS, LIFESCI' ENTERED AT 14:40:24 ON 28 JUN 2005
L1 51978 S ALPHA(W)AMYLASE?
L2 15362 S ASPERGILLUS (W)ORYZAE
L3 2086 S L1 AND L2
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L5 14 S L3 AND L4
L6 13 DUP REM L5 (1 DUPLICATE REMOVED)
L7 68103 S THERMOSTAB?
L8 92 S L3 AND L7
L9 150853 S DOUGH OR BREW OR BEER OR ALCHOHOL OR MALTPOSE
L10 15 S L8 AND L9
L11 11 DUP REM L10 (4 DUPLICATES REMOVED)
L12 811 S L2 AND IMMOBILIZ?
L13 2 S L4 AND L12
E BISGARD-FRANTZEN H/AU
L14 2 S E4
E SVENDSEN A/AU
L15 375 S E3
E PEDERSEN S/AU
L16 1367 S E3
L17 1742 S L14 OR L15 OR L16

=> s l3 and l17
L18 5 L3 AND L17

=> dup rem l18
PROCESSING COMPLETED FOR L18
L19 3 DUP REM L18 (2 DUPLICATES REMOVED)

=> d 1-3 ibib ab

L19 ANSWER 1 OF 3 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN
ACCESSION NUMBER: 2001-12290 BIOTECHDS
TITLE: New variant of Fungamyl-like **alpha-amylase**, useful for production of maltose syrups, includes mutations that improve stability against heat and acidic pH; plasmid pTAKA17 expression in bacterium cell for syrup production, dough improvement, brewing and starch liquefaction
AUTHOR: Bisgard-Frantzen H; SvendSen A; Pedersen S
PATENT ASSIGNEE: Novozymes
LOCATION: Bagsvaerd, Denmark.
PATENT INFO: WO 2001034784 17 May 2001
APPLICATION INFO: WO 2000-DK626 10 Nov 2000
PRIORITY INFO: DK 1999-1617 10 Nov 1999
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2001-367478 [38]
AB A variant (A) of a Fungamyl-like **alpha-amylase** (EC-3.2.1.1) is claimed. (A) has alteration in one of the disclosed amino acid regions. Each alteration is a deletion or substitution of an amino acid and/or insertion of an amino acid downstream of a particular position, and (A) retains **alpha-amylase** activity. Also claimed are: DNA construct (II); recombinant expression vector

(III); a cell (IV) transformed with the (II) or (III); composition for producing high maltose syrup (HMS) or alcohol; dough improving or brewing composition; producing (M1) of liquefied starch, HMS or alcohol using (A); producing (M2) variants of Fungamyl-like enzymes with increased thermostability; production (M3) of (maltose) syrup; and immobilized (A). (A) is used for producing syrups, e.g. of high maltose content, or alcohol from starch, as dough improver for baked goods, in brewing, to increase fermentability of the wort, and for liquefaction of starch.
(47pp)

L19 ANSWER 2 OF 3 EMBASE COPYRIGHT 2005 ELSEVIER INC. ALL RIGHTS RESERVED.
on STN
DUPLICATE 1

ACCESSION NUMBER: 2000374774 EMBASE
TITLE: Expression and characterization of a recombinant *Fusarium* spp. galactose oxidase.
AUTHOR: Xu F.; Golightly E.J.; Schneider P.; Berka R.M.; Brown K.M.; Johnstone J.A.; Baker D.H.; Fuglsang C.C.; Brown S.H.; **Svendsen A.**; Klotz A.V.
CORPORATE SOURCE: F. Xu, Novo Nordisk Biotech, 1445 Drew Avenue, Davis, CA 95616, United States. fengxu@nnbt.com
SOURCE: Applied Biochemistry and Biotechnology - Part A Enzyme Engineering and Biotechnology, (2000) Vol. 88, No. 1-3, pp. 23-32.
Refs: 16
ISSN: 0273-2289 CODEN: ABIBDL
COUNTRY: United States
DOCUMENT TYPE: Journal; Conference Article
FILE SEGMENT: 029 Clinical Biochemistry
LANGUAGE: English
SUMMARY LANGUAGE: English
ENTRY DATE: Entered STN: 20001116
Last Updated on STN: 20001116

AB The *Fusarium* spp. (*Dactylium dendroides*) galactose oxidase was expressed in **Aspergillus oryzae** and *Fusarium venenatum* hosts. Under the control of an *A. niger* **alpha.-amylase** or a *Fusarium* trypsin promoter, high level galactose oxidase expression was achieved. The recombinant oxidase expressed in the *A. oryzae* host was purified and characterized. The purified enzyme had a molecular weight of 66 kDa on sodium dodecyl sulfate-polymerase gel electrophoresis (SDS-PAGE) and 0.4 mol copper atom per mole protein. The stoichiometry increased to 1.2 after a Cu saturation. Based on a peroxidase-coupled assay, the enzyme preparation showed an activity of 440 turnover per second toward D-galactose (0.1 M) at pH 7 and 20°C. The enzyme had an optimal temperature of 60°C at pH 6.0 and an activation free Gibbs energy of 33 kJ/mol. A series of D-galactose derivatives was tested as the reducing substrate for the oxidase. The difference in activity was interpreted by the stereospecificity of the oxidase toward the substituents in the pyranose substrate, particularly on the C5 and the cyclic hemiacetal O sites. The recombinant oxidase could act on some galactose-containing polysaccharides, such as guar gum, but was not able to oxidize several common redox compounds that lacked a primary alcohol functional group.

L19 ANSWER 3 OF 3 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN
ACCESSION NUMBER: 1996-12567 BIOTECHDS
TITLE: New **alpha-amylase** variants;
mutant enzyme construction for improved calcium dependency, substrate binding, cleavage, pH dependent activity and thermostability; application in e.g. surfactant composition
AUTHOR: **Svendsen A**; Bisgard-Frantzen H; Borchert T V
PATENT ASSIGNEE: Novo-Nordisk
LOCATION: Bagsvaerd, Denmark.

PATENT INFO: WO 9623874 8 Aug 1996
APPLICATION INFO: WO 1996-DK57 5 Feb 1996
PRIORITY INFO: DK 1995-1256 10 Nov 1995; DK 1995-128 3 Feb 1995
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 1996-371424 [37]

AB A method for constructing a Termamyl-like **alpha-amylase** (TAA) mutant is new in which the variant has **alpha-amylase** (AA, EC-3.2.1.1) activity and at least one altered property as compared to the parent AA. The method involves: analyzing the structure of TAA to identify an amino acid residue or structural part which alters the property; constructing a TAA variant; and testing the variant for the property. Also claimed are: a method of constructed a variant which has decreased calcium ion dependency, altered pH dependent activity, increased thermostability and reduced ability to cleave a substrate close to the branching point. The variants can be used as surfactants or for desizing or starch liquefaction. They can also be used for the production of sweeteners and ethanol from starch. (171pp)

=> d his
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FILE 'MEDLINE, EMBASE, BIOSIS, BIOTECHDS, SCISEARCH, HCAPLUS, NTIS, LIFESCI' ENTERED AT 14:40:24 ON 28 JUN 2005
L1 51978 S ALPHA(W)AMYLASE?
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L5 14 S L3 AND L4
L6 13 DUP REM L5 (1 DUPLICATE REMOVED)
L7 68103 S THERMOSTAB?
L8 92 S L3 AND L7
L9 150853 S DOUGH OR BREW OR BEER OR ALCHOHOL OR MALTOSE
L10 15 S L8 AND L9
L11 11 DUP REM L10 (4 DUPLICATES REMOVED)
L12 811 S L2 AND IMMOBILIZ?
L13 2 S L4 AND L12
E BISGARD-FRANTZEN H/AU
L14 2 S E4
E SVENDSEN A/AU
L15 375 S E3
E PEDERSEN S/AU
L16 1367 S E3
L17 1742 S L14 OR L15 OR L16
L18 5 S L3 AND L17
L19 3 DUP REM L18 (2 DUPLICATES REMOVED)

| | L # | Hits | Search Text |
|-----------|------------|-------------|--|
| 1 | L1 | 8206 | alpha adj amylase\$2 |
| 2 | L2 | 2409 | aspergillus adj oryzae |
| 3 | L3 | 504 | 11 same 12 |
| 4 | L4 | 158 | fungamyl |
| 5 | L5 | 15 | 13 same 14 |
| 6 | L6 | 887 | "98-110" or "161-167" |
| 7 | L7 | 1 | 15 and 16 |
| 8 | L8 | 66094 | brew or beer or dough or alchohol or maltose |
| 9 | L9 | 23 | 13 same 18 |
| 10 | L10 | 1 | "5989169".pn. |
| 11 | L11 | 11077 | BISGARD-FRANTZEN- HENRIK SVENDSEN PEDERSEN |
| 12 | L13 | 9 | 14 and 112 |
| 13 | L12 | 78 | 13 and 111 |

| | Issue Date | Pages | Document ID | Title |
|----|-------------------|--------------|--------------------|---|
| 1 | 20050512 | 30 | US 20050100996 A1 | Methods for producing ethanol from carbon substrates |
| 2 | 20050127 | 58 | US 20050019886 A1 | Alpha-amylase variants |
| 3 | 20041118 | 17 | US 20040229764 A1 | Fungamyl-like alpha-amylase variants |
| 4 | 20030925 | 28 | US 20030180900 A1 | Methods for producing ethanol from carbon substrates |
| 5 | 20030911 | 64 | US 20030170769 A1 | Alpha-amylase mutants |
| 6 | 20021219 | 6 | US 20020192291 A1 | Amylose products as matrix former for programmed release systems, process for preparing these amylose products, and process for making programmed release systems |
| 7 | 20020523 | 16 | US 20020061476 A1 | Protective overcoat for an imaging element comprising an enzyme-treated biopolymer |
| 8 | 20050118 | 6 | US 6844172 B2 | Amylose products as matrix former for programmed release systems, process for preparing these amylose products, and process for making programmed release systems |
| 9 | 20020827 | 99 | US 6440716 B1 | .alpha.-amylase mutants |
| 10 | 20020618 | 15 | US 6406838 B1 | Protective overcoat for an imaging element comprising an enzyme-treated biopolymer |

| | Issue Date | Pages | Document ID | Title |
|----|-------------------|--------------|--------------------|---|
| 11 | 20020423 | 5 | US 6376219 B1 | Amylose products as matrix former for programmed release systems, process for preparing these amylose products, and process for making programmed release systems |
| 12 | 20010828 | 15 | US 6280912 B1 | Protective overcoat for an imaging element comprising an enzyme-treated biopolymer |
| 13 | 20000208 | 100 | US 6022724 A | .alpha.-amylase mutants |
| 14 | 19991123 | 100 | US 5989169 A | .alpha.-amylase mutants |
| 15 | 19980915 | 5 | US 5807578 A | Fast-melt tablet and method of making same |

| | Issue Date | Pages | Document ID | Title |
|---|------------|-------|-------------------------|--------------------------------------|
| 1 | 20041118 | 17 | US 20040229764 A1 | Fungamyl-like alpha-amylase variants |

| | Issue Date | Pages | Document ID | Title |
|----|-------------------|--------------|--------------------|---|
| 1 | 20050512 | 30 | US 20050100996 A1 | Methods for producing ethanol from carbon substrates |
| 2 | 20041118 | 17 | US 20040229764 A1 | Fungamyl-like alpha-amylase variants |
| 3 | 20041007 | 138 | US 20040197854 A1 | Methods for modifying the production of a polypeptide |
| 4 | 20040909 | 23 | US 20040176317 A1 | Functionalised maltosyl fluoride as glycosyl donor in the chemo-enzymatic preparation of ratio of oligo- or polysaccharides |
| 5 | 20040909 | 131 | US 20040175814 A1 | Novel transferase and amylase, process for producing the enzymes, use thereof, and gene coding for the same |
| 6 | 20040812 | 37 | US 20040157301 A1 | Methods for producing end-products from carbon substrates |
| 7 | 20031030 | 37 | US 20030203454 A1 | Methods for producing end-products from carbon substrates |
| 8 | 20030925 | 28 | US 20030180900 A1 | Methods for producing ethanol from carbon substrates |
| 9 | 20030925 | 34 | US 20030180416 A1 | Carbohydrate oxidase and use thereof in baking |
| 10 | 20030501 | 133 | US 20030082595 A1 | Nucleic acids of aspergillus fumigatus encoding industrial enzymes and methods of use |
| 11 | 20050531 | 30 | US 6900039 B2 | Carbohydrate oxidase and use thereof in baking |
| 12 | 20020521 | 119 | US 6391595 B1 | Transferase and amylase, process for producing the enzymes, use thereof, and gene coding for the same |

| | Issue Date | Pages | Document ID | Title |
|----|-------------------|--------------|--------------------|---|
| 13 | 20011127 | 129 | US 6323002 B1 | Methods for modifying the production of a polypeptide |
| 14 | 20010703 | 5 | US 6254903 B1 | Process for making baked articles that retain freshness |
| 15 | 20010206 | 21 | US 6184011 B1 | Method of releasing solid matrix affinity adsorbed particulates |
| 16 | 20001226 | 30 | US 6165761 A | Carbohydrate oxidase and use thereof in baking |
| 17 | 19990928 | 131 | US 5958727 A | Methods for modifying the production of a polypeptide |
| 18 | 19980630 | 4 | US 5773055 A | Process for preparing a bean flavor |
| 19 | 19961231 | 6 | US 5589207 A | Method of producing a frozen yeast dough product |
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